

BUDDING AND LOCOMOTION IN THE SCYPHISTOMAS OF URELIA

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The Scyphozoa have three principal methods of reproduction: (1) sexual reproduction by means of gametes formed by the free-swimming medusæ; (2) lateral budding by the scyphistomas or polyps which develop from fertilized eggs; and (3) strobilization—a sort of transverse fission by which a scyphistoma gives rise to a series of young medusæ. The studies here reported concern the second of these methods of reproduction; namely, lateral budding. They concern also the closely related phenomenon of locomotion by means of “sterile buds” or pedal stolons.

Lateral budding in a scyphistoma was described one hundred years ago by Dalyell (1834), who also saw strobilization (1836) and noted that after giving off the young medusæ (ephyræ), the basal portion of the strobilizing polyp (strobila) returns to the vegetative or scyphistoma stage. Thus, according to Dalyell, the scyphistoma or “Hydra tuba,” as he termed it, is a perpetual condition, able to maintain itself indefinitely by budding. Hérourard (1908) has described budding and locomotion (“métrotropism”); while a fuller account is given by Pérez (1922); and more recently by Halisch (1933). The present account covers somewhat the same ground, but undertakes by observation and experiment, to discover the causal relations involved. In short, we shall attempt to be explanatory.

MATERIAL

The polyps used were presumably those of *Aurelia*, and were obtained in great abundance from the underside of an old float in a slough not far from Pacific Grove, California. They were obtained also from the hulls of destroyers which had lain at anchor for four years in San Diego Bay. I wish to acknowledge my indebtedness to the two California institutions whose guest I was while making these studies: the Hopkins Marine Station of Stanford University, located at Pacific Grove, and the Scripps Institution of Oceanography of the University of California, located at La Jolla.

A scyphozoan polyp (or scyphistoma) consists of two germ layers, ectoderm and entoderm, separated from one another by a thick layer of gelatinous mesogloea. Its body may be divided for purposes of description into two parts: (a) a body portion proper, terminating distally in an oral disc (peristome) bordered by a circle of tentacles and surrounding the mouth. We may call this terminal portion of the body, for want of a better name, the "hydranth." The lips of the mouth project somewhat, as a proboscis. The body is partially constricted internally by four longitudinal folds of entoderm, the gastric ridges (tænioles). (b) Below the body is the stalk, a simple cylinder which ends in a base of attachment. Commonly there is a small amount of

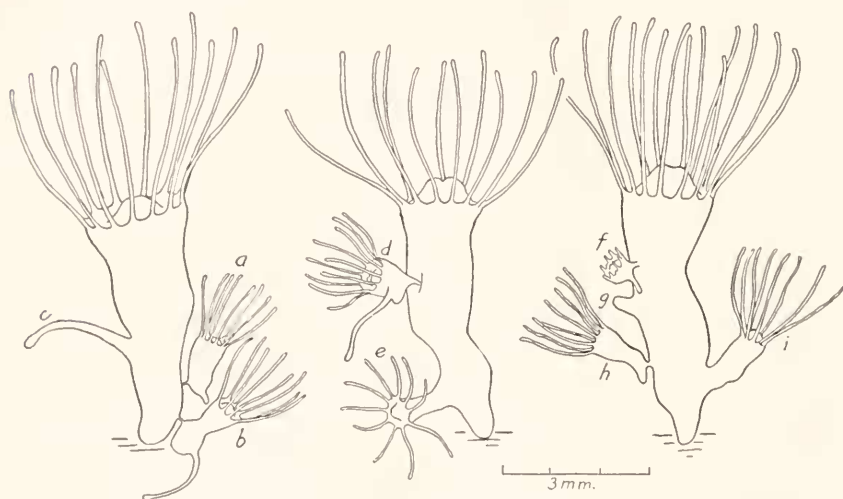


FIG. 1. Scyphozoan polyps (*Aurelia*) showing buds and pedal stolons. *d* and *f* are typical "fig-type" buds. *c* and *g* are pedal stolons. *c* is a "hydrant-type" bud. *a*, *b*, and *h* are intermediate.

transparent secretion about the base; but this is too delicate to serve for attachment.

DESCRIPTIVE

Buds and Pedal Stolons

Buds and stolons develop from the wall of the lower body and stalk. They may be classified as of four principal types; although variation within limits, rather than conformity to rule, is the rule in scyphistomas.

(1) The *fig-type bud* (Fig. 1, *f* and *d*) is an outgrowth of usually the lower body or upper stalk. Typically it is compressed from side to side, and it soon begins to constrict away from the side of the parent.

A mouth and circle of tentacles develop on the side of the bud which is towards the parent's tentacles, while a tendril-like stolon grows out on the side of the bud away from its attachment to the parent. Sometimes the hydranth forms before the stolon appears; sometimes the reverse is the case; but most frequently the tentacles and stolon grow out at about the same time. In the course of a few days the stolon elongates, attaches by its tip, and contracts; thus it draws the bud away from the parent (Fig. 2). A narrow strand of tissue composed of ectoderm only, remains for a time to connect the bud to its place of origin on the side of the parent. Budding of this sort has been described by Perez and Halisch.

(2) A second type of outgrowth is the *pedal stolon* (Fig. 1, *c* and *g*). This is at first a blunt cone, which becomes more and more acute

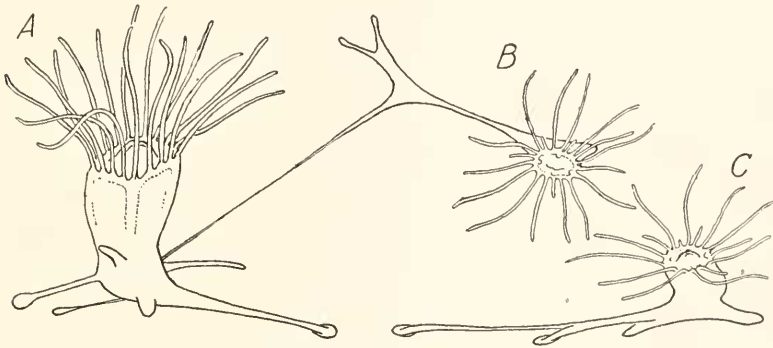


FIG. 2. Locomotion of buds. *A* is an adult polyp with older and younger stolons and from which a bud, *B*, has recently migrated. *C* is another bud migrating.

until it is an elongated tendril of nearly uniform cross-section. Pedal stolons grow almost entirely from the upper stalk portion of the polyp. As a rule they are at first directed outward and upward, that is, away from the polyp's base. Shorter stolons which attach rather promptly may grow downward from the lower part of the stalk, especially in polyps which have been torn loose from their place of attachment.

(3) An intermediate type of outgrowth is the *stolonic-bud* (not figured). This is similar to the pedal stolon in its manner of origin, and usually forms in about the same position. Its distal portion is in fact a pedal stolon; but as it grows outward, its proximal portion draws to itself a more than usual amount of the body wall of the parent polyp. This gradually constricts away from the parent, and as it does so, it forms a mouth and tentacles on the side of the bud near its proximal

end. Sometimes two hydranths are formed side by side, instead of one. The stolon bud differs from the fig-type bud in that the development of the hydranth and the process of constriction away from the parent do not occur until after the stolon has grown out, and even after it has attached.

(4) The fourth sort of outgrowth is the *hydra-type bud* (Fig. 1. *i*, but especially *c*). This is a cylindrical outgrowth, usually from the lower stalk. Its distal end forms a mouth and circle of tentacles. It does not soon form a pedal stolon, but remains attached to the base of the parent for a long time. Indeed, it may grow to be the size of its parent, with the result that small colonies of several individuals are sometimes formed. Ultimately, however, the buds separate by the formation of pedal stolons on the parent or on the bud, above the place of union. The stolon, after attaching and contracting draws the bud and parent apart. When the stolon is on the parent, the body of the parent may be drawn away, leaving the bud attached to the substrate by the old base of the parent. Such a method of separation is described as of regular occurrence in a scyphistoma of unknown genus (Renton, 1930).

The day-by-day history of the fig-type buds, the sequence of tentacle formation, and the descriptive aspects of locomotion by means of pedal stolons have been given by Pérez (1922) and independently by Halisch (1933).

LOCOMOTION

The Method of Locomotion

Pedal stolons serve two purposes: for locomotion and to provide new bases of attachment. The first process is seen to best advantage in the migration of buds away from the parent (Fig. 2). The stolon grows out on the side of the bud away from the parent's body. It then attaches to the substrate, contracts, and so draws the bud after it. Then another stolon grows out on the side of the bud away from the parent; and this in turn lengthens, attaches, and contracts. Thus step by step the bud moves from its place of origin. After perhaps six steps, the stolons appear less frequently and in less organized fashion; and although change of location still takes place, forward locomotion in one direction is not so consistently observed.

Even in adult polyps, however, forward locomotion in one direction is occasionally seen (Fig. 3). A number of polyps were removed from the oyster shells to which they were attached, into a dish of sea water. Individuals were then chosen which showed neither buds nor stolons,

and these were placed in individual dishes. Twenty-four hours later most of them had developed stolons, and again twenty-four hours later most of them had attached. (Possibly isolation favored the formation of stolons, although this is not proven.) Careful drawings were made of certain individuals from day to day; and it was found that some sent out two or more stolons, either simultaneously or in close succession, which thus anchored the polyps to one spot; while the majority formed stolons one by one, roughly at two-day intervals. There was in some instances a tendency for stolons to form successively on the same side, so that a more or less directed locomotion took place.

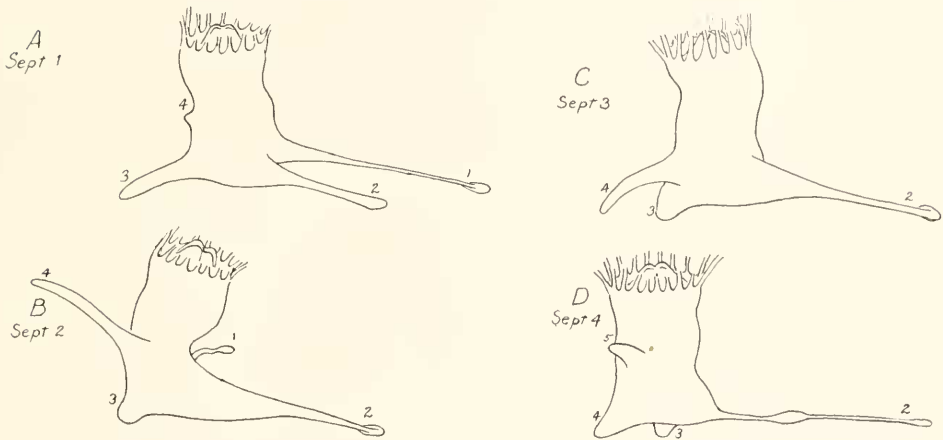


FIG. 3. Locomotion of an adult polyp. The same polyp was drawn on four successive days. The pedicel stolons are numbered from 1 to 5 in the sequence of their formation.

"Life History" of a Pedicel Stolon

Locomotion by stolons will become clearer if we consider the "life history" of a single stolon (Figs. 3 and 4). (a) The stolon begins as a blunt, conical projection from the upper stalk portion of a polyp (Fig. 3A, stolon 4). (b) It then elongates and pushes outward and somewhat upward away from the base of the polyp (Fig. 3B, stolon 4). It continues to grow in length until it has become a tendril-like process composed of an ectodermal sheath with a solid entodermal core, free to wave to and fro in the moving water. Indeed, it apparently has some movement of its own. (c) When fully elongated the tip of the stolon (ectoderm) develops glandular cells and becomes adhesive. Coming into contact with some surface it attaches (Fig. 3C, stolon 4). (d)

Contraction follows immediately, and with so much power that the entire polyp is pulled toward the new point of attachment (Fig. 3*D*, stolon 4). (*c*) The stolon now becomes for a time molded over into the base of the polyp; its attached tip becomes the foot (Fig. 3*D*, stolons 4 and 3). (*f*) In due time another stolon forms, elongates, attaches, and contracts; thus drawing the polyp onward. The old stolon (the base)

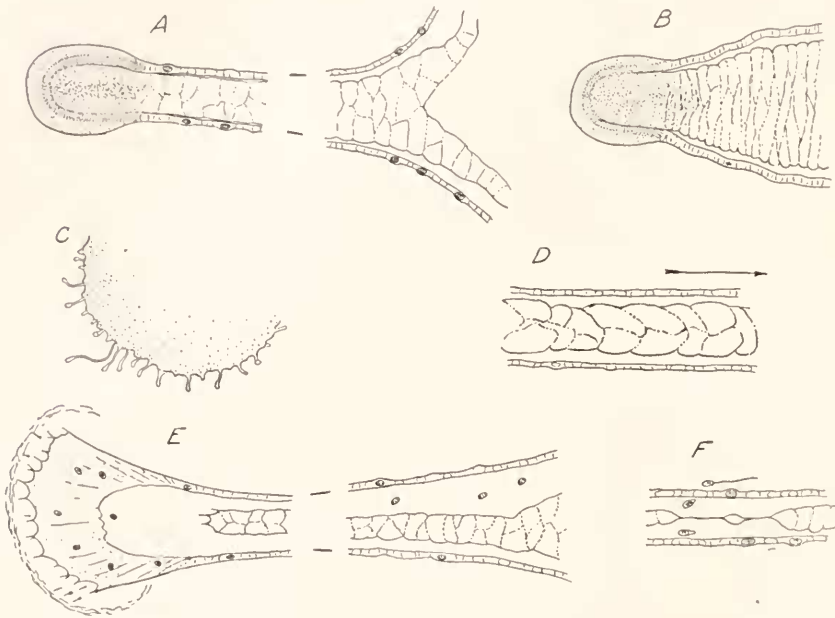


FIG. 4. Living pedal stolons as seen with the high power of the microscope (optical sections). *A*, a young stolon with specialized ectodermal tip and core of entoderm. *B*, the same contracted as a result of mechanical stimulation. *C*, enlarged detail showing margin of a tip undergoing attachment to the substrate. *D*, a stolon under tension following attachment. The core of entoderm is migrating back into the stalk of the polyp in the direction of the arrow. *E*, attached stolon under tension. The adherent tip is anchored to the bottom of the dish by means of fibrils. The core of entoderm is partly withdrawn. *F*, an old stretched stolon. The entoderm has become reduced to a thread with a few cells scattered along it. Cnidoblasts are shown in this and other figures.

is thus stretched out into a thread composed almost solely of ectoderm, which for a time retains its attachment to the substrate (Fig. 3*A* to *D*, stolon 2). (*g*) Soon, however, the old stolon breaks from its attachment, and is withdrawn into the side of the stalk of the polyp, usually with some debris adhering (Fig. 3*A* and *B*, stolon 1). The entire history of a pedal stolon thus suggests strongly the behavior of a pseudopod

of a rhizopod protozoan; except, of course, that the stolon is composed of many cells.

The above description applies when one stolon is formed at a time. When several are formed, each one cannot become a base (Fig. 2*A*). Some develop tension without being able to shorten. After a day or so in this case also the entodermal core is withdrawn; and still later the ectoderm pulls away from its attachment and is withdrawn.

Rôle of the Germ Layers in Locomotion

Observations under the high power of the microscope make it clear that the two germ layers of the polyp play diverse rôles in the processes of budding and locomotion. We shall not discuss their parts in the development of a hydranth at this time, except to note that the entoderm comes very close to the ectoderm in the region where a mouth is forming, and in the regions where tentacles are about to grow out. (*a*) The first visible evidence of a bud or stolon, similarly, is a conical projection of entoderm as seen through the overlying transparent ectoderm. The tip of the entoderm seems to push away the intervening mesogloea and to come into actual contact with the ectoderm. (*b*) As the stolon grows outward a rearrangement of the entoderm takes place (Fig. 4*A*). The entodermal cells of the wall of the stalk migrate actively into the forming process and rearrange themselves into a solid core. The ectoderm, meanwhile, undergoes little change in its appearance and in the arrangement of its cells, except that it grows thinner. (*c*) An exception to the above statement is the ectoderm of the tip of the stolon, which thickens and becomes glandular. It is worth noting again that it is in this region that the ectoderm is in most close contact with the underlying entoderm.

If an elongated stolon be mechanically stimulated, a contraction of the ectodermal sheath takes place (Fig. 4*B*). The tip does not contract, and the entodermal core appears to be only passively compressed. Its cells become disc-shaped, and give the appearance in lateral view of a pile of coins. Contraction of this sort, however, is only temporary; and in a short while (an hour or two) the stolon is again fully elongated.

(*d*) Contact of the tip of the stolon with the substrate stimulates the glandular cells to discharge a cementing substance in the form of fibrils (Fig. 4*C*). On coming into contact with the solid surface the ends of these fibrils adhere firmly and so serve to anchor the tip of the stolon. Hérouard (1911) has described and figured a process of the formation of "tonofibrilles" which differs in some details from the above statement. As this process is taking place the tip of the stolon

spreads out and flattens down in close apposition to the surface. A thin perisarc, a sort of mucus, is secreted around this area of attachment (Fig. 4E). (e) Contraction of the stolon follows. This begins with a flow of the core of entoderm toward the body of the polyp (Fig. 4D). The flow is most rapid in the middle, with the result that the entodermal cells become convex in the direction of the polyp's stalk. As the entoderm is withdrawn, the space between the ectodermal sheath and the entodermal core becomes wider and wider (Fig. 4E). If a stolon should be torn from its mooring at this stage of contraction, a contraction of the ectoderm takes place, and the narrow entodermal core is thrown into a spiral coil within the ectoderm. (f) On reaching the stalk the entodermal cells rearrange themselves again into the entodermal layer of the body wall. This involves, of course, the development of a cavity between them.

(g) As the entodermal core is withdrawn the ectodermal sheath develops tension. If the polyp is free to move, it is pulled toward the new point of attachment, which thus becomes the new base. If, however, the polyp is not free to move by reason of other attachments, the entoderm becomes drawn out into a thin axial thread with a few entodermal cells scattered along it (Fig. 4F). After thus serving for a time as an anchoring line, the ectoderm finally pulls away from its attachment and is withdrawn into the side of the stalk.

Morphallaxis of the Stalk

From the descriptions just given, it will be seen that the stalk portion of a polyp is of remarkable plasticity. It continually changes its form and structure as one stolon after another is formed and resorbed. The process is not to be classed as growth in the usual sense, since it does not involve an increase in the size and number of cells. Rather, the cells change their shape and to some extent their specifications. First, for example, the upper stalk cells become stolon cells with the tendency to form a solid process. Then, after the stolon has attached, they become progressively transformed into stalk cells again. Plastic molding of this sort was termed by Morgan "morphallaxis."

Usually when a stolon attaches and contracts, the stalk becomes bent downward toward the new attachment (Fig. 3B). In recovering its upright position, material is withdrawn (especially entoderm) from the old base and stolon (Fig. 3C and D). Looked at grossly there is thus a flow of materials of the stalk from the side on which old stolons are being resorbed to the side on which new stolons are forming. This movement was demonstrated in an experiment in which spots of vital dye

(Nile blue sulphate) were placed upon the side of the upper stalk. (The polyp was laid on a piece of paper and a crystal of the dye was touched to the surface for a few seconds. The polyp was then returned to sea water and any adhering dye was washed off.) In some instances stolons grew out from the region of the spot, or from just above the spot. It was then observed that the spots elongated into the stolons. Later,

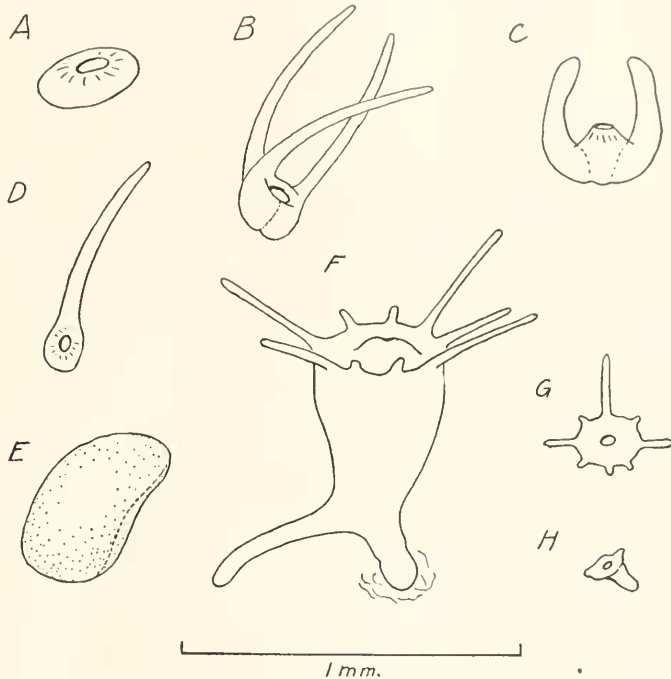


FIG. 5. Reconstitution from small pieces. *A* is a small piece taken from the oral disc (peristome). *B*, *C*, and *D* are tentacles with a small amount of adjacent material from around their base. *E* is a piece of entoderm which has rounded but not undergone regeneration. *F* is a comparable piece of ectoderm. It has formed a complete polyp. *G* and *H* have regenerated from very small fragments of ectoderm.

when the stolons were resorbed, the marked areas again became compact spots, but upon the side of the stalk opposite to that upon which they were originally placed.

EXPERIMENTS

Reconstitution

Regeneration of Small Fragments.—Animals which possess the capacity for vegetative reproduction are as a rule also able to regen-

erate themselves from pieces. This is true of scyphozoan polyps. Indeed, no part of this animal is without some power of restitution, although the power differs from region to region both in degree and in kind.

Small pieces roughly one millimeter square isolated from different locations behave differently. (a) A piece cut from the oral disc (peristome), and including a part of the oral lip but no tentacles, will roll itself together and will regenerate one mouth, sometimes two mouths; but usually no tentacles nor any part of the body below the tentacles are formed (Fig. 5A). (b) A similar piece taken from the region of tentacles will close and regenerate lips and oral disc, sometimes additional tentacles, but usually no body (Fig. 5, B and C). If a tentacle has been cut off it will grow out again. Sometimes the stump of a tentacle will regenerate a bifurcated tentacle. (c) An isolated tentacle heals its cut end but otherwise undergoes little change. It may live for days, swimming about as though it were a ciliated worm. Occasionally a tentacle cut close to its base regenerates a small mouth (Fig. 5D). (d) A piece of the upper body removed from just below the circle of tentacles promptly regenerates an oral disc and tentacles. (e) From the upper body region downward to the attached base there is a gradual decrease in hydranth-forming tendency. The hydranths which are formed at lower levels are smaller, of fewer tentacles, and require a longer time for their regeneration. Considerable variability in hydranth-forming power exists, however, especially at the lower body and upper stalk levels. Some pieces form whole, well-proportioned polyps, and do so quite promptly. Other pieces from the same level form single large stolons, or even two stolons. No doubt this variability reflects the bud-forming or stolon-forming tendencies which were present in this region of the polyp at the time the pieces were isolated. (f) Pieces taken from the basal end of the polyp or near it usually round up into a ball and secrete a loose perisarc about themselves. (g) Isolated stolons commonly attach by their tips and then, after contracting, regenerate entire small polyps (Fig. 9A-D). The gradient of hydranth-forming tendency, which has just been described, is illustrated in the behavior of transverse segments as shown in Fig. 6. Segments *b* and *c*, which are from the upper body, have their hydranths fairly well reformed on the third day. Segments *d*, *e*, and *f*, which are from the lower body and upper stalk have small hydranths on the seventh day. Stolons are most advanced in segments *c*, *d*, and *e*. Segment *g* has rounded up and secreted perisarc. Later it may break from its perisarc and regenerate a very small polyp.

These observations show that invisible differentiation (segregation,

chemo-differentiation, determination) is present in the polyp. No two parts behave in the same fashion when they are isolated. The invisible differentiation is not complete (stable or irreversible determination) except apparently in the oral lips and tentacles. Elsewhere large powers of regulation remain; or in other words, invisible differentiation is incomplete (labile determination), and redifferentiation is possible. Invisible differentiation reveals itself principally in formative tendencies; that is, in hydranth-forming tendencies which are strongest at the upper

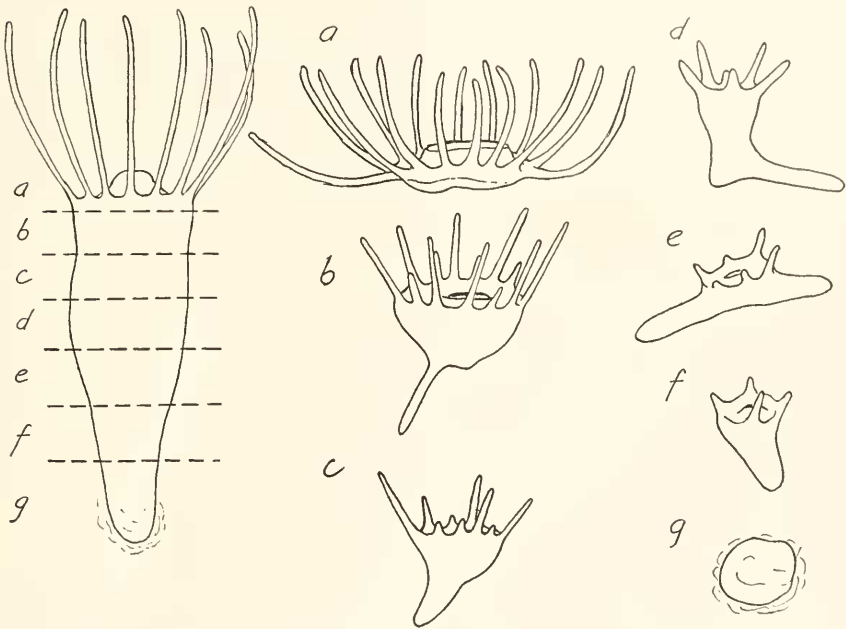


FIG. 6. Reconstitution from transverse segments. Note the decreasing hydranth-forming tendency from *a* to *g*; the pronounced stolon-forming tendency in *c* to *e*; the base-forming behavior in *g*. *a*, *b*, and *c* were drawn on the third day; *d*, on the fourth day; *e*, *f*, and *g* on the seventh day.

end of the polyp and decrease toward the lower end, and in base-forming tendencies which are strongest at the lower end. The power to form typical pedal stolons is greatest in pieces from the upper stalk area.

Polarity in Regeneration

Polarity in the scyphistomas is similar to the same phenomenon in *Hydra* and the hydroids. In general, transverse sections regenerate apico-basally; that is, with the hydranth formed from the upper cut surface (Fig. 6, *b* to *f*). Sometimes, however, sections cut from just

below the circle of tentacles regenerate biapically; that is, with a hydranth from both the upper and the lower cut surfaces (Fig. 7*A, B*, and *C*). This is most likely to occur if the section be thin.

Longitudinal halves and quarters of polyps from which the hydranth has been removed regenerate asymmetric hydranths, which reveal both the inherent polarity of the piece and the orienting effect of wounding. If the piece be long the new hydranth is formed from the upper cut

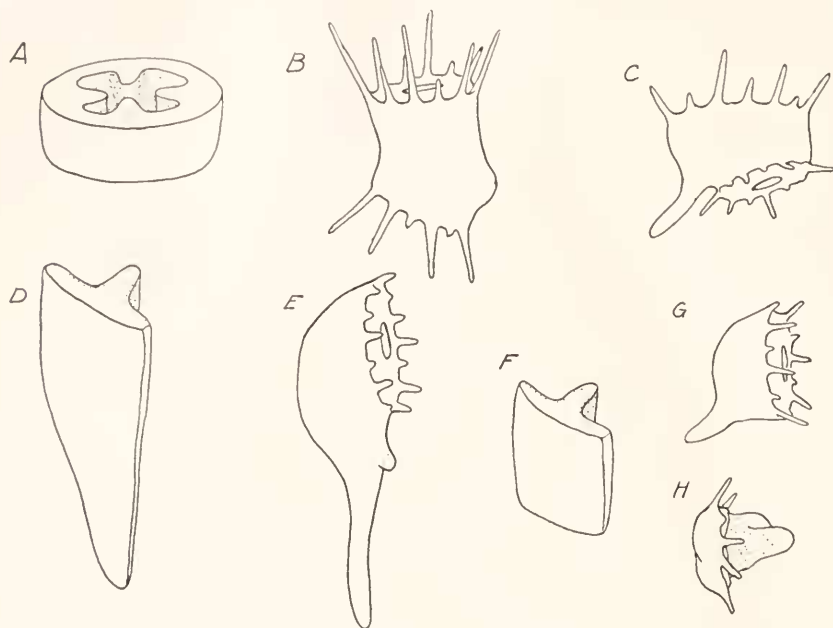


FIG. 7. Polarity in regeneration. *A, B*, and *C*. Thin transverse sections from the upper body region frequently regenerate biapically. *D* and *E*. A longitudinal quarter of a polyp (hydranth removed) regenerates obliquely, that is, with the hydranth facing upward and also inwards. *F* and *G*. A quarter of a body segment regenerates with the hydranth facing inwards, at right angles to the original polar axis. *H*. Similar to *G* except that the large entodermal gastric ridge prevented closure of the wound. Oral lips and tentacles have formed from the margin of the wound.

end; but the hydranth is oblique, facing inward as well as upward (Fig. 7, *D* and *E*). If, however, the piece be short, the new hydranth may be entirely symmetric and face directly inward (Fig. 7, *F* and *G*.) The oral lips and tentacles are in this case formed from the cut margins of the piece. Indeed, the wound may never close, as in cases in which the entoderm of the gastric ridge is disproportionately large (Fig. 7, *H*.)

These facts suggest that polarity in scyphistomas is less a matter of

orientation (for example, of molecules or of "intimate structure") than it is of gradation. The upper body portion is strongly hydranth-forming. Moreover, the hydranth-forming potential is not greatly different between the opposite surfaces of thin pieces taken from just below the tentacles. Were polarity primarily orientation, the proportion of biapical regenerates would not increase as sections are cut thinner. If the basis of polarity is orientation, it must be of a very labile nature, to account, for example, for the change in the direction of polarity which results from wounding.

Unlike the hydranths, the pedal stolons do not form at cut surfaces. In the case of biapical regenerates they form from the side of the piece. In monapical regenerates they form from uninjured body wall just to one side of the healed lower cut surface. In longitudinal pieces the stolons form from the non-wounded surface (see later). It is obvious that stolon formation obeys different laws with respect to polarity and with respect to the effects of wounding, from the laws of hydranth formation.

Regeneration from Ectoderm and Entoderm

It is fortunately possible to separate pieces of ectoderm and entoderm and to observe the behavior of these two germ layers separately in explants. It is then found that entodermal pieces round up into ciliated balls, which may remain alive and rotating for several days, but which do not regenerate (Fig. 5, *E*). Pieces of ectoderm, on the other hand, round up, and within a period of 7 to 11 days regenerate small, complete polyps (Fig. 5, *F*, *G*, and *H*). The evidence indicates that entoderm is irreversibly differentiated as entoderm, whereas ectoderm is labile and dependent for its differentiation. (The reversibility of the ectoderm is presumably due in whole or in part to the presence in ectoderm of so-called interstitial or restitutional cells.) Whether or not the entoderm is regionally differentiated has not been directly determined. There is, however, indirect evidence that it is.

When ectoderm is isolated alone it rounds up and within a few days regenerates fairly well balanced polyps, the number and size of the tentacles of which depend on the size of the piece (Fig. 5, *G* and *H*). The form of the regenerate has little relation to the region of the polyp from which the ectoderm is taken. (Ectodermal explants have not been made from the oral disc, tentacles, or base.) Pieces from the stalk region, however, are somewhat slower regenerating than pieces from the body. When ectoderm and entoderm are isolated together, the regenerates reveal very distinct regional differences, as has already been described. These observations indicate that regional differentiation

exists primarily in the entoderm, and that the ectoderm is largely dependent upon the entoderm for its regional characters.

LOCOMOTION

Experiments on the Origination of Stolons

In the preceding section we noted that the pedal stolons of the scyphistomas typically form from the upper portions of the stalk, and that as they grow outward they tend to point upward away from the base. They also have a tendency to form on the side of the stalk away

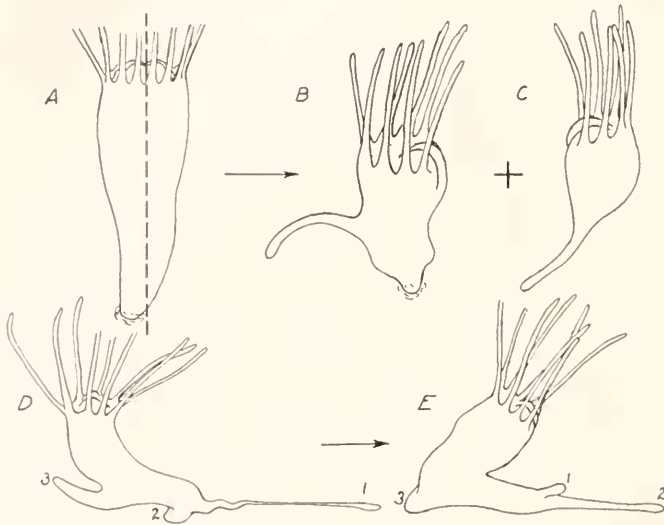


FIG. 8. Pedal stolons formed by half polyps. *A*. A polyp has been cut approximately into halves. *B*. The piece which retains the base forms a typical pedal stolon from the side opposite the cut. *C*. The piece which lacks the base forms a short stolon from near the lower end. *D*. A half polyp in locomotion toward the left. *E*. Later stage of the same polyp.

from the remnants of older stolons. These facts suggest that the base and the older stolons have an inhibiting influence upon the formation of new stolons.

To test this assumption two experiments were performed:

(1) Twenty polyps were selected which showed neither buds nor stolons, and to which no injury had been done. A bit of the calcareous substrate adhering to the base was taken as evidence that the base was intact. From ten of these polyps the base was then cut away. Twenty-four hours later about equal numbers of the operated and the unoperated polyps (five and six respectively) had produced stolons. The rate of stolon formation is therefore not greatly, if at all, influenced by

the removal of the base. But the stolons of the two groups were characteristically different. In all but one instance, the operated animals produced stolons which grew downward from the lower end of the remaining stalk; while the control group produced long, typical pedal stolons which grew upward and outward from the side of the stalk. The experiment was repeated with similar results.

(2) Twenty polyps were again selected as before, but were cut

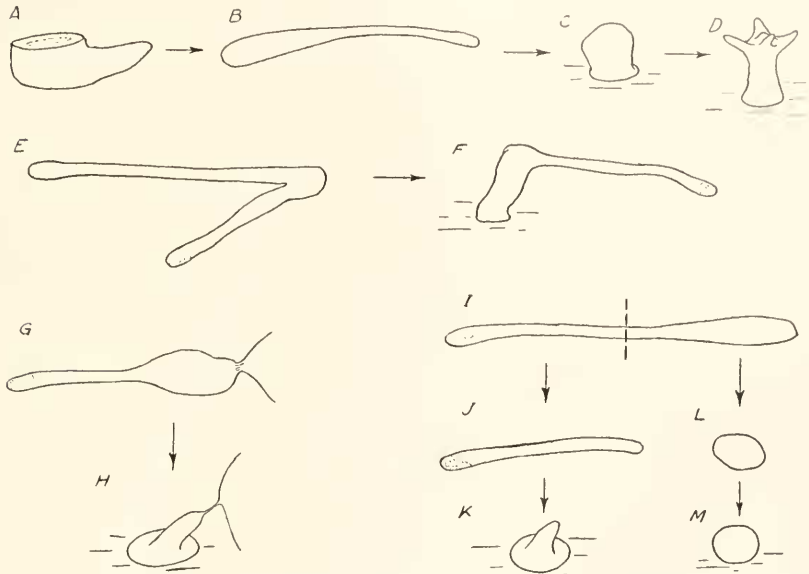


FIG. 9. Operations on pedal stolons. *A*. A section of the stalk including a beginning stolon. *B*, *C*, and *D*. The stolon elongates, attaches, and contracts, and in due time regenerates. *E*. An isolated stolon which has developed a branch stolon at its base. *F*. The original stolon has attached and contracted; but the branch stolon remains unattached and elongated. *G*. A pedal stolon was crushed near its proximal end and immediately contracted in the region of injury (temporary contraction). *H*. Later the tip attached and permanent contraction took place. *I*. An isolated stolon was cut into two pieces. *J* and *K*. The distal piece remained elongated until its tip attached, when it contracted. *L* and *M*. The proximal half immediately rounded into a ball which later adhered lightly to the bottom of the dish.

longitudinally into halves, except that in each instance the cut passed just to one side of the base (Fig. 8, *A*). Forty-eight hours later essentially the same numbers of each group had produced stolons (15 of those with base; 17 of those without). In most cases those without a base had produced short, downward-growing stolons (Fig. 8, *C*); while all those with a base had formed upward and outward-growing stolons (Fig. 8, *B*). Thus again the presence of a base influenced the place of origin and the direction of growth of the stolons.

A further observation in connection with this latter experiment is important: The stolons always grew from the uninjured side of the half polyp. This was not only true of the first stolon, but of several succeeding stolons as well (Fig. 8, *D* and *E*); with the result that the partial polyps locomoted in one direction for a time; namely, in the direction away from the injured side.

These observations may be summarized by saying that (1) the rate at which stolons are formed is little if at all influenced by the presence or absence of a base, or by wounding; but (2) the position and direction of outgrowth of each new stolon is definitely affected. New stolons commonly grow from that portion of the stalk which is farthest from the base, and on the side opposite to the remains of older stolons. New stolons also grow from the side opposite a lateral wound.

Experiments on the Elongation of Stolons

A pedal stolon begins as a blunt cone, first of entoderm, then of entoderm and ectoderm as well. Within the space of a few hours it elongates until it has become a long, thin, tendril-like process with a solid core of entoderm. What processes are involved? We have already observed that elongation is a process of morphallaxis, in which the growing stolon draws to itself material from adjacent regions of the stalk. Experiment confirms this observation, and also indicates the primary rôle of the tip in the process of outgrowth.

(1) In several experiments beginning stolons were cut away along with a greater or less amount of the stalk (Fig. 9, *A*). In most cases the stolon continued to elongate, and in some instances (provided the base was not included) the entire fragment became transformed into a thin stolon of nearly uniform cross-section (Fig. 9, *B*). From this we conclude that the stolon is a "self-differentiating" system, physiologically independent of the polyp proper, and capable of exerting some measure of control over stalk material immediately surrounding the base of the stolon.

(2) In further experiments the tips of elongated stolons were crushed by pinching them with forceps. The stolons immediately contracted; and within the space of a few hours they usually had become completely withdrawn into the material of the polyp stalk. In some instances, however, when the injury was not too great, the tip was able to reorganize itself, or a new tip just proximal to the injured tip was formed. When this occurred the stolons re-elongated. Again, when the tip of a stolon was lightly stained with the vital dye, Nile blue sulphate, it remained extended; but when the staining was heavy, the stolon contracted. In several instances after such a contraction a new

tip developed just proximal to the stained region, and the stolon again elongated.

We conclude from these experiments that the outgrowth of a pedal stolon is a self-determined morphallactic process, independent of the polyp proper, but dependent upon the presence and activity of a free and uninjured tip.

Experiments on the Contraction of Pedal Stolons

The contraction (or retraction) of a stolon normally takes place immediately after its tip has attached to some solid object. If attachment fails to occur, the stolon may remain elongated for two or more days without contracting. The following experiments show that physiological continuity with an unattached tip is necessary if stolons are to remain uncontracted.

(1) It was regularly observed that when stolons are cut away from the polyp proper, they remain elongated until and unless the tips adhere to the substrate. The stimulation which results from injury at the proximal end of the stolon at most produces a slight, temporary contraction of the ectodermal sheath. When the tip adheres to the substrate, however, the isolated stolon immediately and permanently contracts into a ball (Fig. 9, *C*). Control is obviously from the tip. A week or so later the ball will have regenerated a tiny whole polyp with mouth and circle of tentacles (Fig. 9, *D*).

(2) In many instances isolated stolons developed a second tip, usually near their proximal end. When this occurred a branch stolon grew out (Fig. 9, *E*) under the controlling influence of the second tip. It was then noted that when one of the tips attached to the substrate, only that portion which was under the control of that tip contracted. The part under the control of the free tip remained elongated (Fig. 9, *F*).

(3) When isolated stolons are cut into two or three pieces, the distal pieces with their free tips invariably remain elongated until and unless attachment occurs (Fig. 9, *I*, *J*, and *K*). (The few exceptions were presumably due to injury to the tip.) The proximal and intermediate pieces, on the other hand, invariably round up into balls (Fig. 9, *I*, *L*, and *M*). Occasionally after rounding up, such a piece may develop a tip and then re-elongate; but in every case observed the time interval was long (at least twenty-four hours), and the phenomenon was clearly one of regeneration.

(4) When an elongated stolon is crushed by a pair of forceps at some point between the tip and its base, a wave of contraction spreads in both directions from the point of injury. The contraction which

moves toward the tip is temporary (it is apparently of ectoderm only), and indeed it never quite reaches the tip (Fig. 9, *G*). In a short time this distal portion is again elongated. The contraction which moves proximally, on the contrary, is permanent. (It presumably is a temporary ectodermal contraction followed by complete ectodermal and entodermal retraction.) A day later the proximal portion, in the case of isolated stolons, is rounded into a ball. In the case of stolons which have not been cut away from the polyp, the portion proximal to the cut becomes completely resorbed into the stalk, while the distal part may dangle as a loose appendage connected with the polyp by a thin filament of ectoderm only. In Fig. 9, *H* such a piece has attached to the substrate and contracted.

(5) Ligatures of fine thread were tied about elongated stolons, breaking the continuity of the entodermal core but not of the ectodermal sheath. It was then observed that the portions of the stolons proximal to the ligatures promptly contracted, and in many instances succeeded in drawing the distal portion through the ligature and into the wall of the polyp stalk. Any portion of the stolon which remained distal to the ligature, however, continued elongated.

These several experiments plainly indicate that the control within the stolon is a one-way control, that it is of the tip over more proximal levels, and that physiological continuity, especially of the entoderm, is essential if the stolon is to remain elongated. It is further noted that neither the stimulus of wounding, nor the effects of healing of the wound, have a permanent effect upon the stolon in producing contraction. Only the attachment of the tip, or injury to the tip, or the removal of the tip is effective.

It has been emphasized that the presence of a free tip is necessary if the stolon is to remain elongated. However, if the cut be clean and very close to the end of the stolon, so that a part of specialized ectoderm of the tip remains as a part of the stolon, the stolon may in some instances remain elongated and in due time attach and contract normally. It thus appears that the functional "tip" of the stolon is the terminal glandular region of the ectoderm.

DISCUSSION

General Formative Principles

The polyps which we have just described come as near to being the plastic "candle flames" and "whirl pools" which Thomas Henry Huxley and others have discussed as any objects of animate nature, certainly as any objects among the Metazoa. However, they are no

without a considerable degree of stability of structure. The upper body possesses a definite morphology which is relatively constant. The lower body and stalk, although plastic and changing, nevertheless perform according to certain general principles. Still, it cannot be said that these principles are always rigidly obeyed.

As a first principle we note that although the outgrowths which take place from the lower body and stalk possess one or the other or both of two potentialities; namely, the power to form hydranths and the power to form stolons; yet these two potentialities are not realized to the same extent and in the same way at the different levels of the polyp proper.

(a) The power to form a hydranth may be realized at any level below the polyp's hydranth. In the lower body and upper stalk, however, there is a sort of opposition between the parent hydranth and the bud hydranth, which leads to the bud promptly pinching itself away from the parent (fig-type of bud), and retaining its connection with the parent by only a thin strand of ectoderm. When the differentiation of a bud hydranth is delayed (stolonic buds), the pinching away from the parent is also delayed. In the lower stalk region no such opposition exists, and the buds which are formed here (hydra-type buds) long remain attached to the parent by a broad, fleshy union. Intermediate conditions are to be found at intermediate levels.

The experiments upon the regeneration of fragments show that the power to form hydranths is greatest in the material immediately below the circle of tentacles; yet buds do not normally form here. It is obvious that the hydranth of a polyp inhibits the formation of a bud and so of a second hydranth in the very region where the tendency to form hydranths in explants is strongest.

(b) The potentiality of stolon production is most pronounced in the upper portion of the stalk. It is here that the more typical stolons are produced; and it is here also that the buds (fig-type) are most certain to form stolons. In the lower stalk region there is an opposition or incompatibility between the polyp proper and the production of stolons, with the result that but few stolons are produced, and these are not typical stolons, but short processes which attach quickly. The buds of this region (hydra-type buds), moreover, are mostly without stolons.

Why does this incompatibility exist? A first hypothesis is that the base and the older stolons exert some sort of inhibitory influence upon the production of new stolons. Against this view, however, is the fact that the removal of the base has little if any influence on the rate at which new stolons form. A second possible hypothesis is that the entoderm in the region where new stolons form is in a sense "younger"

entoderm, that is, it has not been involved in stolon-forming activity for a longer period of time.

In brief, the location and structure of the several types of buds and stolons illustrate a principle of opposition and toleration: opposition between parent hydranth and the bud hydranth, and between the base and the stolon; toleration, however, of hydranth for stolon, and of base for buds. Buds and stolons develop most frequently at intermediate levels because the material of these intermediate levels is less differentiated either hydranth-ward or base-ward than that of the upper and lower levels.

The Rôle of the Germ Layers

A second principle to be noted concerns the parts played by the two germ layers in budding and locomotion. The entoderm appears to play the independent rôle. Its cells rearrange themselves in characteristic fashion as the bud develops or as the stolon forms and elongates. The ectoderm appears to be more passive, responding perhaps to some sort of inductive stimulus of the entoderm, especially where the ectoderm is in close contact with the entoderm; or adapting itself to the form assumed by the entoderm. Except in the tentacles, at the oral lips, and at the tips of stolons little specialization of the structure of the ectoderm is to be observed.

There is experimental evidence that the entoderm is the principal seat of invisible differentiation. When small pieces of ectoderm from the body or stalk are isolated they constitute themselves into whole and fairly well-balanced polyps, and they do so quite irrespective of whether they are from upper or lower levels. Similar pieces of entoderm show a higher degree of differentiation by not regenerating. Pieces which are composed of both ectoderm and entoderm, however, regenerate, but show strong regional differences, both qualitative and quantitative.

Buds

Why do buds arise? There is no answer more satisfactory than that given by Child—that they arise as a result of physiological isolation when the region which becomes the bud escapes from the dominating and inhibiting activity of the parental hydranth. They do not arise near the upper end because the inhibitory activity of the parental hydranth is strongest here. They do not arise at the lower end because the hydranth-forming power is weakest here. They arise at an intermediate level. The pinching away of the bud from the parent's body accompanies the differentiation of the bud hydranth.

An important feature to note is that the pinching away of the bud is at first complete only so far as the entoderm is concerned. The bud may retain its connection with the parent by a strand of ectoderm for a considerable time. The morphogenetic correlations seem to take place within the entoderm.

Pedal Stolons

Origination.—The problem of the origin and behavior of pedal stolons is of special interest. The polyps produce pedal stolons (also buds) one after another more or less continuously. Isolated pieces sooner or later produce stolons. Why then do stolons originate? The answer is not "polarity," for the stolons usually form at intermediate levels, and in explants they sometimes arise very close to the hydranth. It seems as though something accumulates in less differentiated regions which leads to the production of stolons.

The earliest sign of a pedal stolon is a cone of entoderm on the side of the stalk, which pushes outwardly, displacing the intervening mesogloea, and comes into close contact with the surface ectoderm. As soon as this occurs the ectoderm responds by becoming the "tip" of an outgrowing stolon. One is inclined to interpret the process as an "induction" comparable to that by which, in amphibian development, the chorda-mesoderm of the primary archenteric roof induces overlying ectoderm to become the neural plate. However, critical experiments upon this phase of stolon development have not yet been performed.

Elongation.—As soon as a specialized tip is present the stolon elongates; and it does so by drawing to itself material from the stalk. Having elongated, it continues in this state, so long as the tip is free, so long as it is uninjured, and so long as physiological continuity remains between the tip and the remainder of the stolon. Something which the tip does produces elongation. What may be the nature of this action?

A first suggestion is that the tip dominates in the physiological manner which Child has frequently described. The pedal stolon is then to be compared with a bud; its tip is the apex of the bud. According to Child a bud originates when some region on the side of the parent's body becomes so increased in its rate of physiological activity that it becomes "physiologically isolated" from the individuating forces of the remainder of the animal. The region then begins to grow away from the parent and to differentiate into a new individual. Child (1929) was able to produce such a bud experimentally in the hydroid *Corymorpha*, a form which normally never produces buds in nature, by merely wounding the side of the stalk of the polyp. The first sugges-

tion, then, is that the outgrowth of a pedal stolon is an example of physiological isolation and comparable to budding. The tip of the stolon is physiologically dominant, the remainder of the stolon subordinate.

Several observations indicate against this hypothesis and emphasize the contrast rather than the similarity between the origin of buds and the origin of pedal stolons.

(1) The tip of a stolon never differentiates into the apex of a polyp; that is, into a hydranth. Indeed, the opposite occurs. When a stolon is cut away and attaches by its tip, it is very apt to regenerate a small hydranth; but in this case the apex of the hydranth is always formed at the proximal end of the stolon. In fig-type buds, similarly, the physiologically dominant apex of the bud becomes the oral region of a hydranth; but the stolon is an outgrowth from the side of the bud of a different physiological nature.

(2) A hydranth may be produced by the stimulation of wounding, but stimulation or wounding never produces stolons. Again, the opposite is the case. When a polyp is divided longitudinally, the stolons always form from the uninjured surface of the pieces. Moreover, stimulation or injury to the tip of an elongated stolon results in the immediate retraction of the stolon.

(3) The development of a hydranth seems to depend upon some general and quantitative property of the cells which produce it, such as a high rate of metabolism; but the development of a pedal stolon depends upon the presence of a definite specialized region which we have called the tip. So long as a part of this specialized tip is present, elongation continues; but if the specialized tip is removed, contraction takes place.

A second hypothesis to account for the control which the tip of a pedal stolon exerts over the rest of the stolon is that the tip is an organ of internal secretion and produces a hormone, which, diffusing into the entoderm, causes the latter to organize itself as the central core of a stolon. This hypothesis recalls the activity of the growth hormone of plants, auxin. It has been found that in plants the growing tips of most stems, petioles, flower stalks, and coleoptiles produce a substance which, diffusing from the tip into the regions immediately below, induce the cells of the latter to elongate in a longitudinal direction (see Went, 1935). May it not be that something comparable to this takes place in the pedal stolon? May we not suppose that the specialized glandular cells of the ectodermal "tip" of the stolon act as an organ of internal secretion and synthesize a "growth hormone" which diffusing into the entoderm causes the cells of the latter to arrange themselves into a

solid core? May we suppose that this internal secretion of the hormone continues until the specialized cells come into contact with a solid surface (or are injured) and discharge their cementing substance to the outside? Internal secretion then ceases, the hormone supply is cut off, and the elongated stolon is retracted.

This hormonal hypothesis seems to cover the facts. However, before it can be more than a mere working hypothesis, it will be necessary to know more concerning the structure and interrelations of the germ layers at the tip of the stolon; and grafting experiments will have to be performed.

If we may provisionally accept this hormonal hypothesis, then it follows that within the polyp we have two different physiological types of control: (a) Within the hydranth we have dominance and subordination of the sort which Child has described. In relation to a gradient-field which is established, the localization and differentiation of the zones of the hydranth is accomplished. Dominance of this sort is not due to a specific influence emanating from the functional apex, but rather to the establishment of a labile configuration of forces with the apex as its center (see Gilchrist, 1937). (b) We have secondly, by hypothesis, hormonal control within the pedal stolon. The early differentiation of one small region as a specialized organ of internal secretion results in a definite arrangement of the cells in the surrounding area, and a stolon is produced.

Both controlling regions, the lips of the mouth and the tip of the pedal stolon may be termed apices or "centers of organization"; but the apex of the hydranth dominates because of its high rate of physiological activity and its influence on the configuration of forces in the gradient-field; while the apex of the stolon controls because of the activity of a specific product of its metabolic activity. The first type of control is quantitative and dynamic; the second qualitative and chemical. The first, to employ Child's (1921) terminology, is transmissive; the second is transportive. Moreover, the differentiation of the hydranth and the development of the stolon are different sorts of processes. The first is a relatively irreversible process in which cells become specialized as oral lips, peristome, or tentacles. The formation of a stolon, on the contrary, is entirely reversible. It is a temporary arrangement of the nature of a morphallaxis, which persists only so long as the hormonal control emanating from the tip persists.

Contraction.—We have described two different types of contraction, which we may designate as temporary and permanent contraction. Temporary contraction takes place when a stolon is mechanically stimulated, as by pricking it with a needle or pinching it with forceps. The

wave of response is then seen to move in both directions from the point of injury, although the wave which moves distally may never reach the tip. Temporary contraction of this sort is never complete, and is apparently of the ectoderm only. Within the space of an hour or so the stolon is again fully elongated.

Permanent contraction, or better, retraction, is of an entirely different nature. It takes place normally when the tip of a stolon attaches to the substrate. Experimentally it may be produced by injuring or removing the tip, or indeed by merely breaking the physiological continuity by means of a ligature. The first evidence of such contraction is seen in the movement of the cells of the entodermal core of the stolon toward the stalk of the polyp. Permanent contraction is best thought of as a negative process; a sort of undoing of the factors which produce elongation. In terms of the hypothesis of a growth hormone, permanent contraction results from the disappearance of the hormone.

The ectoderm is active in physiological (that is, temporary) contraction. Certainly in the stolon, the entodermal core appears to be passively compressed or thrown into a spiral when the stolon is stimulated. The entoderm, on the other hand, appears to play the more active rôle in morphogenetic (permanent) contraction. It plays the primary part also in the plastic straightening into an upright position of the stalk of a polyp after it has been bent down as a result of stolon attachment. In these morphogenetic changes of form the ectoderm appears to be passive, or at least to do little else than contract and thus supply tension.

SUMMARY

The processes of budding and locomotion have been studied in a scyphozoan polyp, presumably the scyphistomas of *Aurelia*. It has been found (1) that the buds which develop near the hydranth of the polyp are quick to pinch away from the polyp (except for a connecting strand of ectoderm) and to migrate away by means of pedal stolons; whereas the buds which develop near the polyp's base may long remain attached. This indicates an opposition between the hydranth of the polyp and the bud.

(2) The stolons which develop at the upper end of the stalk of the polyp are quite commonly organs of locomotion as well as of attachment. They elongate, attach by their tips, contract, and so draw the polyp forward. The new stolon becomes the new base of the polyp, while the old base becomes drawn out and finally breaks from its attachment. The fact that new stolons form away from the base and away

from the older stolons indicates an opposition between base and young stolons.

(3) The entoderm apparently plays the primary rôle in the formation and differentiation of buds, and in the formation, contraction, and final resorption of pedal stolons. Entodermal cells of the stalk of the polyp rearrange themselves into a solid core as the stolon elongates; they actively migrate back into the stalk as the stolon contracts.

(4) Small pieces taken from any region of the polyp show some power of regeneration. In general, there is a decline in hydranth-forming potentiality from the upper to the lower end. Pieces from the hydranth (oral disc and circle of tentacles) are irreversibly determined to form parts of a hydranth. Pieces from the body or stalk may regenerate whole polyps, although the relative size of the regenerated hydranth decreases as the base is approached. Base-forming tendency is strongest at the lower end. The power to form pedal stolons is greatest in pieces from the upper stalk.

(5) Small pieces of ectoderm only may round up and regenerate whole polyps. Pieces of entoderm round up but do not regenerate. Pieces including both ectoderm and entoderm regenerate in a manner typical of the region from which they are taken. The entoderm is thus the seat of irreversible invisible differentiation (chemo-differentiation).

(6) Various operations were performed on pedal stolons and upon the polyps which produce them; such as fragmentations of the polyp, and injury, isolation, fragmentation, and ligation of the stolons. The results indicate (*a*) that new stolons commonly grow from portions of the polyp's stalk farthest from the base and opposite the remains of older stolons, also on the side opposite a lateral wound; (*b*) that the outgrowth of a stolon is a self-determined morphallactic process independent of the polyp proper but dependent upon the presence and activity of a free and uninjured "tip"; (*c*) that contraction immediately follows in a part of a stolon when the physiological continuity between the part and the tip is interrupted.

(7) It is suggested that the formation of the specialized ectodermal tip of a stolon is the result of an induction originating in underlying entoderm; and that the tip having thus originated acts as an organ of internal secretion in producing a "growth hormone." The hormone diffusing into the entoderm causes the entodermal cells to arrange themselves as the solid core of a stolon. The secretion of the hormone ceases when the specialized cells of the tip come into contact with the substrate and discharge externally.

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